Discovery of novel 5-HT2A receptor agonists with non-hallucinogenic potential and translational antidepressant drug-like profiles

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INTRODUCTION

TREATMENT-RESISTANT DEPRESSION

- Impacts approximately 30% of patients with major depressive disorder.[1]
- Currently, there are limited treatment options, and long-lasting (>2 weeks) treatments are not yet available.

PSYCHEDELICS

- Chemically diverse group of 5-HT2AR agonists, such as psilocybin and LSD.
- Clinically, they show **rapid** and **lasting antidepressant efficacy** following single dose.[2,3]
- Preclinically, antidepressant mechanisms may include promotion of **neuroplasticity**.[4, 5]

CHALLENGES

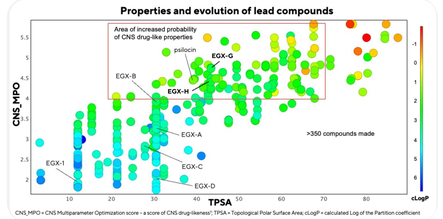
- **Hallucinatory effects** of psychedelics require administration in a controlled clinical setting and exclude certain patient populations.
- Concomitant **agonism of 5-HT2BR** by non-selective psychedelics may lead to valvulopathy risk with frequent use.[6]

APPROACH

GOAL

Novel non-hallucinogenic selective 5-HT2AR agonist for TRD

AI/ML-driven drug design + Medicinal chemistry (SAR)



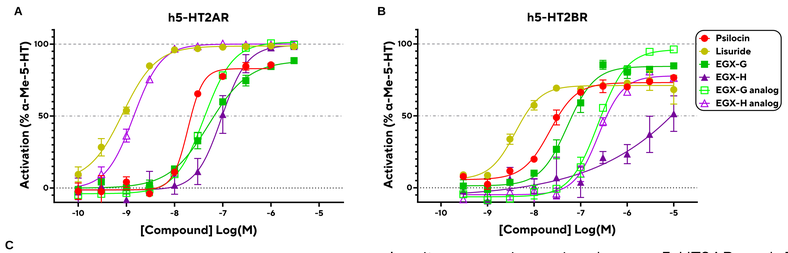
1) 5-HT2AR over 5-HT2BR agonist selectivity

2) Non-hallucinogenic potential

3) Antidepressant-like activity

1) 5-HT2AR OVER 5-HT2BR AGONIST SELECTIVITY

EGX compounds exhibit improved 5-HT2AR over 5-HT2BR selectivity



In vitro screening using human 5-HT2AR and 5-HT2BR agonism IP1 accumulation HTRF assays guides structural activity relationship (SAR) understanding (A and B, respectively).

EGX-G and EGX-H are hits from distinct chemical series with promising 5-HT2AR agonist potency and/or selectivity over 5-HT2BR (C, Table 1). Ongoing SAR analyses continue to identify potent analogs with improved 5-HT2AR agonist selectivity (EGX-H Series), compared to reference hallucinogenic and non-hallucinogenic compounds (C, Table 1).

*Relative Selectivity = $10^{(5HT2A\ Relative\ Agonism\ -5HT2B\ Relative\ Agonism)}$;
Relative Agonism = $\left(\log\left(\frac{Test\ Compound\ Emax}{Test\ Compound\ EC50}\right)\right) - \left(\log\left(\frac{Control\ Emax}{Control\ EC50}\right)\right)$;
Reference Agonist = α-Me-5-HT

Table 1. 5-HT2AR & 5-HT2BR Agonism Readouts of EGX, Psilocin & Reference Non-hallucinogenic Compounds

Human Target (Readout)	EGX-G	EGX-H	Psilocin	Lisuride	2-Br-LSD
5-HT2A (Gq-IP1); EC50 (nM) [Emax]	51.3 [89%]	**92.8 [100%]**	18.2 [83%]	0.83 [99%]*	*0.81 [60%]†*
5-HT2B (Gq-IP1); EC50 (nM) [Emax]	51.9 [84%]	**2,522 [51%]**	21.6 [73%]	3.91 [71%]*	*>10,000†*

*Aequorin Ca++ Mobilization Agonist Readouts 5-HT2A: EC50 = 378nM, Emax = 77%; 5-HT2B: EC50 >10uM; †Gq dissociation BRET assay.

Further agonist profiling at 5-HT receptor subtypes revealed **distinct pharmacological profiles,** compared to reference hallucinogenic and non-hallucinogenic compounds (Table 2).

Table 2. Other 5-HT Receptor^ Agonism Readouts of EGX, Psilocin & Reference Non-hallucinogenic Compounds

Human Target (Readout)	EGX-G	EGX-H	Psilocin	Lisuride	2-Br-LSD
5-HT2A (Arrestin); EC50 (nM) [Emax]	**119** [35%]	**172 [84%]**	27.8 [41%]	*15.3 [44%][10]*	*0.73 [38%][8]*
5-HT2C (Gq-Ca++); EC50 (nM) [Emax]	5.56 [93%]	**112** [82%]	11.9 [105%]	*7.76 [75%]9**	*3.85 [46%][8]†*
5-HT1A (Gi-cAMP); EC50 (nM) [Emax]	**369 [74%]**	**17,150 [105%]**	2,053 [40%]	*1.26 [98%]9**	*11.3 [73%][8]‡*
5-HT1B (Gi-cAMP); EC50 (nM) [Emax]	<5 [100%]	**>100,000**	<5 [100%]	*26.3 [85%]9**	*5.28 [84%][8]‡*

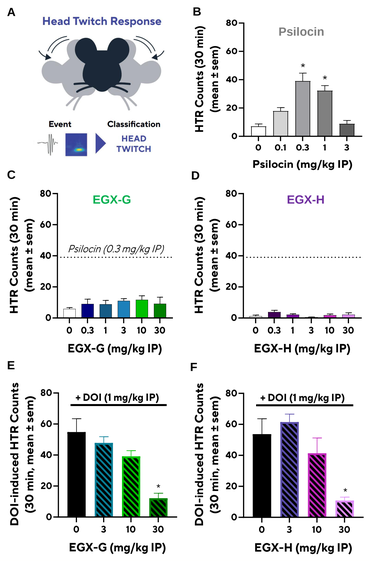
^Targets of potential relevance to antidepressant-like effects and/or expression of HTR; *[35S]GTPγS assay; †Gq dissociation BRET assay; ‡GoM dissociation BRET assay.

2) NON-HALLUCINOGENIC POTENTIAL

EGX-G and EGX-H do not induce Head Twitch Response (HTR) and attenuate DOI-induced HTR

HTR is a behavioral proxy for human hallucinogenic potency (A). To investigate hallucinogenic potential, male C57BL/6 mice, each implanted with a cranium-attached magnet, were administered EGX compounds, placed individually in a glass cylinder surrounded by a magnetometer, and the HTR was measured for 30 min.

Psilocin, a known hallucinogen, significantly induced HTR (B). Neither EGX-G (C) nor EGX-H (D) up to 30 mg/kg induced HTR, suggesting a lack of hallucinogenic potential. Moreover, compound pretreatment (60 min) reduced HTR induced by a known hallucinogen, DOI, in a dose-dependent manner (E, F). These data are indicative of 5-HT2A receptor interactions *in vivo*.



Conclusions

EGX-G and EGX-H are promising starting points for discovery of novel non-hallucinogenic 5-HT2AR agonist antidepressants that may exhibit durable efficacy and potential for flexible dosing options in a broad patient population.

- In vitro, they are potent 5-HT2AR agonists with pharmacological profiles distinct from reference hallucinogenic and non-hallucinogenic compounds. EGX-H exhibits 5-HT2AR over 5-HT2BR agonist selectivity with potential for improved cardiac safety.
- In vivo, they demonstrate attenuation of DOI-induced HTR without inducing HTR on their own, indicating in vivo 5-HT2AR interactions and non-hallucinogenic potential. Both compounds show translational antidepressant-like activity.

Future Directions

- Enhanced in vitro profiling to improve SAR understanding related to hallucinogenic potential and efficacy (e.g., receptor downstream signaling, neuroplasticity assays).
- Continued optimization of novel, potent and selective 5-HT2AR agonist lead molecules (e.g., increase oral bioavailability).

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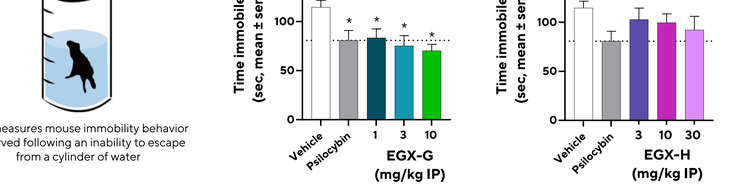
3) ANTIDEPRESSANT-LIKE ACTIVITY *IN VIVO*

EGX-G attenuates immobility in the mouse Forced Swim Test

Antidepressant-like properties of EGX compounds were measured in the Forced Swim Test (A). Male C57BL/6 mice were injected with test compounds and 24h later placed individually in a cylinder of water, where immobility was analyzed for 4 min.

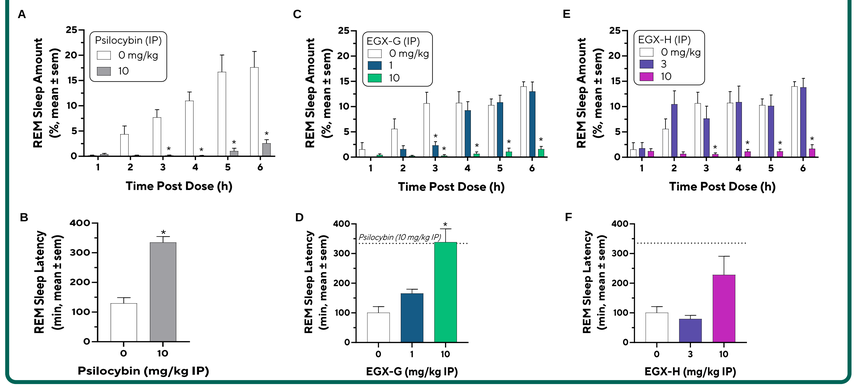
EGX-G significantly reduced immobility at all doses tested (B), similar to psilocybin (5 mg/kg, B, C), indicative of antidepressant-like effects. In contrast, EGX-H (up to 30 mg/kg) did not significantly reduce immobility 24h after dosing (C).



EGX-G and EGX-H show antidepressant-like effects in Wistar Kyoto rats

Translational antidepressant potential was tested in male Wistar Kyoto rats, which exhibit depression-like phenotypes, including increased rapid eye movement (REM) sleep. REM sleep was measured by EEG and EMG electrodes for 6 hours following administration of compounds.

EGX-G and EGX-H (10 mg/kg) suppressed REM sleep by significantly reducing REM sleep amount (C, E), mimicking the effects of psilocybin (A). Additionally, EGX-G significantly increased REM sleep latency (D), similar to psilocybin (B). EGX-H showed a trend toward increased REM sleep latency (F, p=0.1).



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